Università di Firenze
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Enrico Avanzi
C.I.R.A.A.

II Workshop di Ecofisiologia vegetale

LA FLUORESCENZA DELLA CLOROFILLA: DALLA TEORIA ALLA (BUONA) PRATICA

2nd Plant Ecophysiology Workshop

CHLOROPHYLL FLUORESCENCE: FROM THEORY TO (GOOD) PRACTICE

Concetti e applicazioni dei metodi indagine della fluorescenza della clorofilla a negli studi degli stress delle piante. Implicazioni per l'agricoltura, l'ambiente e non solo.

Concepts and applications of investigation methods of chlorophyll a fluorescence in the study of plant stresses.
Implications for agriculture, environment and much more.

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25-26 MAGGIO 2009

RIASSUNTI DEI LAVORI
BOOK OF ABSTRACTS

PRESENTAZIONI ORALI

ORAL PRESENTATIONS

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10:30 F. Bussotti *et al.*: Responses to ozone of *Populus* clones, assessed with fast kinetic and modulated fluorescence

10:45 M. Piccotto e M. Tretiach: Effetti degli NO_X sui licheni: una questione di *Non-Photochemical Quenching*

pausa

11:45 E. Salvatori *et al.*: Fluorescenza fogliare e stress idrico in specie vegetali: meccanismi di risposta e potenzialità applicative

12:00 A. Dayyoub *et al.*: A model of the functional relationship between photosynthesis and ambient chlorophyll fluorescence

12:15 R. Colombo e M. Meroni: Misura della fluorescenza della clorofilla indotta dal sole con telerilevamento di prossimità

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15:15 L. Guidi *et al.*: Effects of boron and salinity stress on chlorophyll fluorescence parameters of tomato leaves

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15:45 V. Baldassarre *et al.*: Uso della fluorescenza della clorofilla *a* per la valutazione della qualità negli ortaggi da foglia in post-raccolta

16:00 M. Piccotto *et al.*: Attivazione – idratazione dipendente del PSII nei licheni. Uno studio in campo attraverso la fluorimetria diretta e modulata

16:15 M. Sighicelli *et al.*: *Brassica oleracea-Phoma lingam* interaction by means of Imaging PAM Fluorescence

16:30 M. Pollastrini *et al.*: Photosynthetic regulation in leaves of grapevine exposed to different light and UV regimes

THE ANALYSIS OF THE FLUORESCENCE TRANSIENT OJIP: THEORY AND APPLICATIVE POTENTIALS

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Fantasy and Rigor in Physics to define Non-Photochemical-Quench (NPQ) Expressions

If new experimental signals are detected, then they have to be communicated to the scientific community. However very often this new "mysterious" measured and reproducible signal is a trace drawn by an instrument if a sample has been treated in a well defined way. Haw can we call it? Mostly we do not understand why this signal behaves this way. That means we do not know exactly how to call it. If we give a name according of what we think it could be, without having strong arguments or even proofs, then we risk that soon a wrong name will have be given and widely accepted.

To change it on the basis of new "new signals" is a difficult undertaking, because the same erroneous story may start again. The practice shows, that less the name of a new signal says what it means, longer will be its life time. More you call the signal, by how to create it or how it looks and not what it means, then this description may remain quasi for ever. P700 or P680 or C550 as light induced absorption changes are names for facts every body can test and provoke with an appropriate equipment. It's meaning can change from school to school. To label the fast fluorescence rise in an alphabetical order (from slower to faster times) as F G H I J K L O (1) or for the main visible steps from shorter to longer time as F_O F_J F_I F_P or simply as OJIP-transient (2) is a pure phenomenological fantasy nomenclature. The advantage is that such names are easy to write and as long as the usual alphabet does not change they may last a long time.

The nomenclature was given in analogy to the KLMN conformations, Rhodopsine undergoes upon illumination (3). On the basis of phenomenological fantasy nomenclature we start to construct parameters which express biophysical meanings. Such new defined, in a first approach, as fantasy expressions can become very useful to get an idea of what is going on in our sample if such parameters can be derived from the experimental signals. However if the original signal has already been expressed in a commonly accepted physical language, based on the used biophysical theory behind, then all fantasy expressions can be translated into the standard physical terms, which allows e.g. to compare expressions like fluorescence signals F with expressions like qN or qP or NPQ or any quantum yield etc in terms of conventional terms like: Concentration (of chlorophyll in excited state), of light energy absorption flux (ABS), of the de-excitation rate constants for photochemical and non-photochemical events k_N and k_P and for the relative variable fluorescence of PS II as a function of the fraction of closed (B) or open (A=1–B) reaction centres. Therefore, remaining on a theoretically very simple level, all fluorescence signals and many defined fantasy expressions can be derived with the biophysical terms such as ABS, k_N (and its components) , k_P and B for any environmental conditions (4).

A glossary will show, how most old and new fantasy expressions can be changed by permutations into the three biophysical expressions for: 1) ABS *k_F, 2) k_P/k_N and 3) B. the fraction of closed reaction centres of PS II. For a review see ref. (5).

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Responses to ozone on *Populus* clones, assessed with fast kinetic and modulated fluorescence

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Several *Populus* clones are known to be ozone sensitive, and are used as "model" to study the physiological responses and metabolic pathways to plants subjected to oxidative stress. Among the techniques and parameters suitable to individuate stress conditions, those related to the fluorescence of the chlorophyll have several advantages, because they are fast, non destructive, non expensive and very informative. In this presentation we summarize the main results obtained from different experimental activities, in order to evidence the informative potential of direct (JIP-test) and modulated fluorescence. The JIP-test was applied within the experiments carried out in the open-top chamber facilities at Curno (North Italy), in the 2004-2005 period.

Ozone-induced stress were characterized by the inactivation of Reaction Centers (RCs). RCs act as dissipator centers, also called heat sink centers: instead of performing primary photochemistry, they reduce Q_A to Q_A^-. This leads to an increase of the apparent antenna size (ABS/RC). The maximum quantum yield of primary photochemistry in the dark-adapted state (F_V/F_M) displayed only slight variations. Plant responses were not proportional to ozone exposure, and only at the end of the growing season (late July to September), efficiency and performance parameters showed a sudden drop. The shape of the fluorescence transient (FT) – normalized between F_0 and F_M, and between F_0 and F_J – shows very evident peaks at steps K, J and I between F_0 and F_J. These peaks were recorded in ozone-exposed plants at the end of the growing season; but, in some cases, they were evident also at the beginning of the growing season (early warning effect). Each peak corresponds to specific biochemical events. K indicates the reduced efficiency in the water splitting system (OES). J indicates a Q_A accumulation in the single turnover region. Finally, the I to P step seems to be more specifically connected to ozone stress and reflects modifications in the reduction of electron end-acceptors, e.g. Ferredoxin and NADP, which are sensitive to the biochemical properties of FNR and Rubisco. The modulated fluorescence was applied to investigate the effects of ozone on the electron transport efficiency and chlorophyll fluorescence quenching coefficients in two poplar clones showing phenomenological differences in response to ozone chronic fumigation (60 ppb of ozone for 5 h day^{-1} for 15 consecutive days).

At the end of treatment, the effective photosynthetic quantum yield of photosystem II (PSII), as indicated by the ratio of variable to maximum chlorophyll fluorescence, and the fluorescence quenching (qP), that reflects the capacity of PSII reaction centers to compete for chlorophyll excited states and is related to the redox states of Q_A, significantly declined in both clones. Another, ozone increased the non-photochemical quenching coefficients (qNP) indicating that non-radiative dissipative mechanisms are involved in dissipation of excess energy. In particular, after 15 days of ozone treatment, the resistant clone was unable to down-regulate the PSII reaction centers and so an irreversible modification of PSII occurred, rendering qNP irreversible. This result showed that photo-inhibition is caused by transformation of active reaction centers into photochemically inactive centers that dissipate excitation energy into heat, thus causing non-photochemical fluorescence quenching.

Effetti degli NO_X sui licheni: una questione di *Non-Photochemical Quenching*

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Gli NO_X hanno un notevole impatto sull'ambiente e sono inoltre implicati nei processi di formazione dell'ozono atmosferico e dello smog fotochimico. I licheni sono organismi estremamente reattivi alla presenza di sostanze che alterano la normale composizione atmosferica e studi di carattere floristico hanno rilevato un progressivo deterioramento della flora lichenica in concomitanza di elevate concentrazioni ambientali di NO_X.

I meccanismi molecolari di azione degli NO_X su questi organismi sono però ancora poco conosciuti. Uno degli obiettivi delle ricerche effettuate nel nostro laboratorio dal 2005 al 2008 è stato quello di individuare i possibili effetti degli NO_X sui licheni attraverso studi di fluorimetria modulata e numerosi altri parametri fisiologici (scambi gassosi, contenuto di pigmenti fotosintetici liposolubili, conduttività, ecc.). Il lavoro è stato svolto conducendo alcune articolate sperimentazioni utilizzando trapianti, in siti urbani inquinati e non, in condizioni altamente standardizzate, che hanno permesso di verificare gli effetti degli NO_X a concentrazioni ambientali anche in presenza di altri stressori.

Tali ricerche hanno dimostrato che la tolleranza dei licheni agli NO_X dipende strettamente dalla loro ecologia e che l'arido microclima urbano può rappresentare un importante fattore di stress, che modula la risposta delle specie. F_v/F_m, la massima efficienza di conversione quantica del PSII, è più sensibile al protrarsi della disidratazione, indotta dalla *heat island* urbana, che non all'azione di questi gas fitotossici.

Il parametro NPQ, dipendente dalla dissipazione dell'energia assorbita sotto forma di calore, si è dimostrato particolarmente informativo, in quanto risente sia dalla concentrazione che dal tempo di esposizione agli NO_X. Vengono infine discussi alcuni aspetti metodologici che supportano l'uso della fluorescenza clorofilliana nel campo del biomonitoraggio ambientale**.**

Fluorescenza fogliare e stress idrico in specie vegetali: meccanismi di risposta e potenzialità applicative

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Lo stress idrico è uno dei principali fattori limitanti la produttività delle specie vegetali, particolarmente nelle regioni a clima mediterraneo (Asensio et al., 2007). Numerosi studi (es. Epron et al., 1992; Manes et al., 2001) hanno investigato i meccanismi fisiologici attraverso cui le piante rispondono a questo stress, evidenziando come le reazioni della fase luminosa della fotosintesi vengano influenzate a causa delle alterazioni sia funzionali che strutturali che si verificano a livello del fotosistema II (PSII). Tali alterazioni sono evidenziabili attraverso lo studio della cinetica di emissione della fluorescenza della Clorofilla "a" *in vivo*, una tecnica non distruttiva e non invasiva che consente di monitorare lo stato funzionale dell'apparato fotosintetico, ed evidenziare l'effetto dello stress prima che avvengano danni macroscopici a livello dell'organo fogliare (Strasser et al., 1995). La misura della fluorescenza del PSII viene condotta utilizzando apparecchiature ottico-elettroniche – fluorimetri – distinti in due tipologie: fluorimetri non modulati, che misurano la cosiddetta "fluorescenza diretta", e fluorimetri ad impulsi modulati, sviluppati per la misura della fluorescenza "modulata". Il presente lavoro si propone di: 1) valutare l'applicabilità di indici di fluorescenza diretta al monitoraggio dello stato di stress della vegetazione naturale, attraverso l'analisi di un dataset ecofisiologico multitemporale riguardante specie arboree in condizioni di campo; 2) confrontare la sensibilità delle tecniche di fluorescenza diretta e modulata nello studio della performance fotosintetica di individui sottoposti a stress idrico, tramite un esperimento in ambiente controllato effettuato su una specie "modello" (*Zea mays* L.).

Gli studi di campo sono stati condotti su individui di *Quercus ilex* L., *Quercus cerris* L., e *Fraxinus ornus* Ten. nel territorio del Parco Nazionale del Circeo (LT), durante gli anni 2003, 2005 e 2006, caratterizzati da differenti condizioni climatiche. Le misure di fluorescenza diretta sono state effettuate con lo strumento PEA2 (Hansatech Instruments Ltd, UK), parallelamente alle misure di scambi gassosi (fotosintesi netta, conduttanza stomatica e traspirazione fogliare) e potenziale idrico fogliare prima dell'alba ("pre dawn"). I parametri di fluorescenza calcolati tramite il test JIP (Strasser et al., 2000), evidenziano la differente risposta delle tre specie ai fattori ambientali: in particolare, nel leccio il processo fotosintetico risulta influenzato principalmente dalla temperatura (Bussotti, 2004), mentre cerro e orniello risultano maggiormente sensibili allo stress idrico (Manes et al., 2006; Nardini et al., 2003). E' interessante notare come dall'insieme dei dati raccolti sulle tre specie sia emersa una relazione (R^2=0.41) tra il potenziale idrico fogliare "pre dawn" e l'Indice di Performance Fotosintetica (PI_{ABS}) (Strasser et al., 2000); ciò sta ad indicare come PI_{ABS} rappresenti un indicatore piuttosto sensibile alle condizioni di stress idrico, e possa pertanto essere utilizzato per il monitoraggio della vegetazione in campo.

L'esperimento in ambiente controllato (camere climatiche) è stato effettuato su individui di *Zea mays* divisi in due set sperimentali: un set di controllo, irrigato per tutta la durata dell'esperimento, e un set trattato in cui, dopo il completo sviluppo della 8°-9° foglia, l'irrigazione è stata interrotta per 6 giorni. Sono state effettuate misure di scambi gassosi, fluorescenza fogliare diretta (strumento PEA2) e modulata (strumento FMS2, Hansatech Instruments Ltd, UK). I principali risultati mostrano come entrambe le tecniche siano in grado di evidenziare precocemente le limitazioni del processo fotosintetico indotte dallo stress idrico; tuttavia, la fluorescenza non modulata rappresenta un approccio più efficiente che, a parità di misure effettuate, è in grado di fornire un maggior numero di informazioni sugli eventi che si verificano a livello dell'apparato fotochimico.

A model of the functional relationship between photosynthesis and ambient chlorophyll fluorescence

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Chlorophyll fluorescence measurements generally rely on the application of pulses of saturating light for the estimation of PSII photochemical yield and electron transport rates. The use of fluorescence measurements under ambient light, however, would be desirable in order to extend the applicability of the technique to passive remote sensing applications.

Here we present a novel functional model of the interactions between PSII fluorescence and photochemistry, and of the resulting link with photosynthetic rates.

According to the model, the relationship between fluorescence yield and PSII photochemical yield shows a segmented pattern, with a positive association under CO_2-limited conditions and a negative linear relationship under light-limited conditions. A strong and consistent correlation is predicted between PSII electron transport and fluorescence radiance.

The model has been successfully tested against leaf-level measurements under variable [CO_2] and light conditions on individual leaves of *Arbutus unedo*, a schlerophyllous Mediterranean species.

Estimation of solar induced chlorophyll fluorescence with remote sensing techniques

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Interest in remote sensing (RS) of solar-induced chlorophyll fluorescence (F) by terrestrial vegetation is motivated by the link of F to photosynthetic efficiency which could be exploited for large scale monitoring of plant status and functioning. Today, passive RS of solar-induced chlorophyll F is feasible with different prototypes and commercial ground-based, airborne, and even space-borne instruments under certain conditions. This interest is generating an increasing number of research projects linking F and RS, such as the development of new F remote retrieval techniques, the understanding of the link between the F signal and vegetation physiology, and the feasibility of a satellite mission specifically designed for F monitoring. This presentation reviews the main issues to be addressed for estimating F from RS observations in two applications: plant status and photosynthesis.

There are some fundamental challenges in plant status assessment using solar induced fluorescence. The main factors that alter the F signal are bidirectional effects, background response, atmosphere and variability of structural parameters. Therefore is important to have a method for minimizing the effects of green biomass/chlorophyll on the recorded signal. In fact, the intensity of the fluorescence signal at canopy level depends both on the efficiency of the photosynthetic apparatus and from the chlorophyll content. Therefore, in order to correctly interpret the fluorescence signal in terms of photosynthetic efficiency indicator it is necessary to know the chlorophyll content of the investigated target. Canopy with different photosynthetic efficiency and chlorophyll content can lead to the same fluorescence signal. To investigate how fluorescence is sensitive to biomass we conducted a cutting experiment in corn. That is, we acquired data in a progressive thinning experiment in which we removed 6 plants at each time.

Fluorescence can be also evaluated for monitoring ecosystem Gross Primary Production (GPP) along the growing season. We have conducted a two year experiment in rice field during which we collected eddy data and spectral data.

What we learn in these experiment is that fluorescence can be successfully used for early stress detection and to track leaf and canopy level GPP along the growing season. Finally, applications of the measured F signal at the three scales of observation (ground, aircraft and satellite) are presented and discussed to provide the state of the art in F estimation.

Chlorophyll fluorescence as index of plant flavonoid content: in lab and in field applications

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Flavonoids (FLAV) are compounds of the plant secondary metabolism that play fundamental roles in the physiology of vegetation. They protect against harmful UV radiation and oxidative stresses, as well as against pathogens and herbivores. Because of their antioxidant properties FLAV are largely employed in medicine and represent an added quality value for fruit, vegetables and valuable derived products such olive oil and wine.

In this context, the development of spectroscopic methods aimed to the evaluation of FLAV in plant tissues, more conveniently than the costly and time-consuming analysis of compound extracts, is of particular importance.

Anthocyanins, a coloured class of FLAV, are particularly suitable for in situ optical detection since they accumulate in the outer layers of samples and strongly absorb visible radiation. On the other hand, other FLAV have absorption spectra in the UV region, where the reflectance (and even more transmittance) signals of vegetable tissues are too low to allow for the application of classical spectroscopic techniques.

During the last decade a new fluorescence spectroscopic method for the non-destructive assessment of UV-absorbing compounds in leaves have been developed. It is based on the comparison of chlorophyll fluorescence signals excited at different excitation wavelengths.

Compounds accumulated on the external layers of plant organs screen the radiation reaching the chloroplasts in the inner layers by different extent according to their absorption spectrum. Consequently, the chlorophyll fluorescence (ChlF) signals measured at different excitation wavelengths will contain information on the concentration of particular compounds in the organ surface.

The method finds application in the agro-food sector for the quality control of fruits and in eco-physiology studies to monitor the vegetation stress status.

The present talk will include: i) a description of the technique; ii) presentation of portable sensors for in-field applications; iii) examples of measurements, such as flavonol determination in *Triticum aestivum* L. leaves as index of nitrogen deficiency, or in *Vitis vinifera* L. in response to different light exposure; anthocyanin determination in fruits (*Vitis vinifera* L. and *Olea europaea* L.) as maturation index, both as in lab and in field detections.

Effects of boron and salinity stress on chlorophyll fluorescence parameters of tomato leaves

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Many studies have been done about the effect of NaCl salinity on plants grown in closed hydroponics, but less attention has been paid to study how high concentration of trace elements, such as boron (B), can affects greenhouse crops. So the aim of this work was the study of the response of tomato grown in presence of low and high irrigation water NaCl salinity and B concentrations. Tomato plants were grown in perlite bags with recycling nutrient solution, prepared using raw water with two NaCl (0.5 or 10.0 mmol L^{-1}) and B (46 or 185 µmol/L, roughly 0.5 and 2.0 mg/L) concentrations.

Laboratory analysis highlighted a B toxicity resulted in evident chlorosis and necrosis of leaf edges and tips. All the treatments showed a strong difference in B concentrations between marginal and central areas of the leaves, the latter remaining green and viable even in condition of severe B toxicity, as indicated by the determination of chlorophyll fluorescence.

In particular, analyzing the symptomatic and asymptomatic areas of the leaves, the parameters of chlorophyll fluorescence showed strong differences. Even from the analysis of chlorophyll fluorescence imaging, a reduction of the photosynthetic activity in symptomatic areas of leaves in plants treated with high B concentrations was observed. Among the treatments, the saline water and low B is the only one without symptoms and it shows fluorescence values typical of healthy tissues over the whole leaf. Therefore, the protective effect of salinity against B cannot be ascribed to a reduction of its uptake associated to decreased water flux; it basically results from the increased frequency of the nutrient solution discharge in high-NaCl treatments, which reduced the accumulation of B in the root zone.

Chlorophyll fluorescence quenching as a tool to screen olive cultivars tolerant to drought stress

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The measurements of fluorescence chlorophyll is considered a promising technique to rapidly quantify the response to physiological stress in higher plants.

Drought stress can be considered one of the most frequent environmental constraints causing the failure of newly planted trees. Olive tree (*Olea europaea*), a representative drought stress tolerant plant, is one of the most typical and economically relevant plant species grown in the Mediterranean area. However, as different cultivars may exhibit different drought tolerance level, the selection of the most drought tolerant cultivars acquires relevance.

The objective of this study was to determine whether information obtained with chlorophyll fluorescence measurements carried out on detached olive leaves subjected to dehydration *in vitro,* may be translated on the whole olive plant.

Results revealed that *in vitro* measurements were effective to evidence strong differences in the F_v/F_m ratio decline among the cultivars, following 24 hours of dehydration, and it was possible to distinguish different level of putative tolerances. Measurements carried out on whole plants of different cultivars confirmed, indeed, the results obtained *in vitro*. The results indicated that the chlorophyll fluorescence measurement represents a valid technique for a rapid screening of olive cultivars tolerance to drought stress.

Uso della fluorescenza della clorofilla *a* per la valutazione della qualità negli ortaggi da foglia in postraccolta

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La qualità degli ortaggi da foglia è definita da caratteristiche estetiche e nutrizionali. Durante la fase post-raccolta le proprietà qualitative degli ortaggi possono essere rapidamente alterate dalle condizioni di conservazione. La temperatura e il tempo di conservazione influenzano fortemente il periodo di vendita. L'uso di sistemi rapidi e non distruttivi può essere un valido strumento di supporto per garantire la qualità al consumatore. La clorofilla è un pigmento fogliare che diminuisce in post-raccolta ed influenza la qualità estetica del prodotto. In particolare la misurazione della fluorescenza emessa dalla clorofilla *a* del fotosistema II potrebbe essere un ottimo sistema di controllo della qualità degli ortaggi durante la filiera dal produttore al consumatore. Negli ultimi anni si sono diffusi gli ortaggi da quarta gamma che possiedo una vita post-raccolta limitata a 6 giorni. Lo scopo del presente lavoro è stato di valutare la senescenza attraverso la funzionalità della foglia mediante la determinazione della fluorescenza della clorofilla a. I prodotti sono stati conservati a 4 e 10 °C e oltre alla fluorescenza della clorofilla, tra i principali parametri qualitativi, stati determinati la clorofilla, i carotenoidi, gli antociani e i fenoli. I dati ottenuti dalle misure della fluorescenza hanno permesso di effettuare analisi di correlazione e regressione tra i parametri qualitativi e il tempo di conservazione per ogni temperatura utilizzata.

I risultati ottenuti hanno mostrato un'alta correlazione tra il tempo di conservazione e i parametri ricavabili dalla curva d'induzione della fluorescenza. In futuro l'uso di uno di questi indici potrebbe essere utilizzato per monitorare la temperatura di conservazione e/o di trasporto in qualunque punto della filiera e di valutare il grado di senescenza del prodotto prima della vendita.

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Use of chlorophyll a fluorescence for evaluating postharvest quality of leafy vegetables

The leafy vegetables quality is defined by aesthetic and nutritional characteristics. During postharvest stage the qualitative properties of leafy vegetables may be rapidly affected by storage conditions. Storage temperature and duration strongly influence the shelf life. The use of rapid and no destructive methods can be valid system for guarantee the product quality to consumers. The chlorophyll is a leaf pigment that declines in postharvest and affects the product appearance. The chlorophyll a fluorescence can be a good quality marker for evaluating the vegetables quality during the distribution chain. In the recent years the minimally leafy vegetables production has been increased with a shelf life limited to six days. The aim of this work is to evaluate the leaf senescence through the leaf functionality by chlorophyll a fluorescence measurements. Leafy vegetables were stored at 4 or 10 °C and during storage along with chlorophyll a fluorescence also were measured chlorophyll, carotenoids, anthocyanins and phenols. Chlorophyll a fluorescence data were correlated with quality parameters and storage duration at the two storage temperatures. Data obtained showed a high correlation among storage duration and parameters calculated from the fluorescence induction curves. After further validation one of the indexes studied can be used for monitoring the storage/transportation temperature or the senescence stage of leaf vegetables in any point of the distribution chain.

Attivazione – idratazione dipendente del PSII nei licheni. Uno studio di campo attraverso la fluorimetria diretta e modulata

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Il lavoro si colloca all'interno del progetto FISR MICENA "Modello integrato per l'evoluzione degli ecosistemi naturali e agricoli in relazione ai cambiamenti climatici nell'area mediterranea". L'obiettivo della ricerca è stato verificare se le misure di fluorescenza (Chl_aF) diretta e modulata evidenziano coerentemente l'attivazione idratazione-dipendente del PSII del fotobionte lichenico. Nei licheni in stato de-idratato, la fotosintesi è soppressa e l'emissione di Chl_aF è ridotta di ca. 10 volte rispetto allo stato idratato. Gli studi fluorimetrici di campo su questi organismi sono scarsi e ciò è dovuto soprattutto alla mancanza di un protocollo standardizzato e alla scarsa comprensione delle variabili che maggiormente influiscono sulla Chl_aF.

Lo studio è stato condotto su 5 specie di licheni foliosi con fotobionte congenerico (*Trebouxia* spp.), ma con caratteristiche ecologiche differenti. I talli sono stati selezionati in 5 siti di campionamento nella Sardegna Occidentale, localizzati lungo un gradiente di umidità atmosferica. Il protocollo sperimentale ha previsto un pre-trattamento, svolto le sere prima delle misurazioni, durante il quale i licheni sono stati idratati e adattati al buio. Le mattine seguenti sono state effettuate misure di F_v/F_m con un Handy-PEA (JIP test) (Hansatech Instr., UK) e con mini-PAM (Walz, Germany) utilizzando differenti combinazioni di sensibilità strumentale (i.e. *gain*) (n = 600).

I risultati evidenziano come la disponibilità idrica annuale e giornaliera moduli l'attività del PSII di questi organismi. Emergono alcuni aspetti problematici come (*i*) la necessità di regolare il settaggio strumentale in base alla fotofilia dell'organismo e (*ii*) il controllo dell'errore strumentale, indotto della temperatura ambientale, sulle misure dell'emissione di Chl_aF *per sé* (p. es. F_0, F_m).

Vengono quindi proposte delle soluzioni alle problematiche incontrate estendibili anche alle misure di Chl_aF condotte in campo su altri organismi fotoautotrofi.

Brassica oleracea-Phoma lingam interaction by means of Imaging PAM Fluorescence

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The pathogens attack can affect directly or indirectly plant photosynthetic performance and modify leaves optical and fluorescence properties. Many studies showed that under stress conditions the photosynthetic quantum conversion decreases while heat emission and chlorophyll fluorescence increase. Chlorophyll fluorescence emission is an efficient and non-destructive tool to detect stress in the photosynthetic apparatus. The possibility to use the imaging technique allow to highlight early plant-pathogen interaction on whole surface when the spatial variation, due to no uniform alteration in plant metabolism, is occurred.

In this study, early diagnosis and non-destructive monitoring of blackleg disease caused by *Phoma lingam* on broccoli (*Brassica olearacea* var. *italica*) plants was investigated.

Fungi inoculation was carried out on three distinct part of plant: cotyledon, stem and leaf. For each kind of inoculation leaves photochemical parameters were collected at different day both in fungi-infected and uninfected plants. Measuring of fluorescence (Fv/Fm, Y[PSII]), photochemical (q_p) and non-photochemical quenching (NPQ) parameters were performed by the portable Imaging-PAM Chlorophyll Fluorescence fluorometer (Walz, Germany).

Fluorescence images and parameters showed different plant responses relative to spatial and temporal variations in function of fungi inoculation. Imaging analysis have allowed to visualize heterogeneity in plant response. Photochemical parameters changes, even if the symptoms are not evident, were observed. Further areas of infection, corresponding to disease development, were evident only in imaging analysis.

The early and non-destructive diagnosis of disease was confirmed. Therefore, Imaging PAM Fluorescence allows the mapping and the physiological study of host-pathogen interaction.

Photosynthetic regulation in leaves of grapevine exposed to different light and UV regimes

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In the Mediterranean environment plants are subject to high solar irradiance. An excess of sunlight energy, exceeding the amount used for photochemistry reactions, could lead to an over-excitation of the reaction centers with consequent photoinibition damage.

The aim of this study is to evaluate the photosynthetic efficiency in different light environments. The research was carried out in the experimental farm Mondeggi Lapeggi, located in the northern part of the Chianti region in Tuscany. It was conducted at the end of the 2007 vegetative season.

Photosynthetic efficiency at different light regimes was analyzed in two years old plants of grapevine (*Vitis vinifera* L. cultivar Sangiovese) cultivated in pots. Three different light intensity treatments were carried out: 10% of full sunlight, 50% of full sunlight, and full sunlight exposition. The reduction of solar irradiance that received by plants was obtained using shading nets (90% and 50% of shading) fixed on tunnels.

Microclimate under and outside the tunnels was constantly monitored and the solar radiative regimes were characterized through spectroradiometric measurements.

To evaluate the responses to the different wavelengths, UV light filters were also arranged above of vines.

Chlorophyll *a* fluorescence under different light conditions was measured by an HandyPea fluorimeter in four completely expanded leaves for plant. Five plants for each light treatment (three level of shading and two UV conditions) were measured. Before each measurement, leaves were dark-adapted with leaf clips. We carried out, also, the anlysis of the chlorophyll content of leaves and morphometric parameters (leaf mass per area, leaf density, thickness).

The photosynthetic capacity of grapevine in relation to different light conditions was detected with JIP-test.The plants grown in the lower light intensity and without UV radiation (10% of sunlight, with UV filter) showed high photosynthetic efficiency: these plants use the low light intensity available with high trapping capacity, high electron transport efficiency and reduced dissipation. In the plants exposed to full sunlight, the assence of UV radiation lead an increase of parameters involved in the dissipation processes. Plants at the 50% of full sunlight display an intermediate behavior between 10% of sunlight and full light condition.

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Effect of irrigation and nitrogen fertilization on photosynthetic process in fiber sorghum leaves

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Effects of water stress on plant physiology, including leaf photosynthesis, have been extensively studied. Water deficiency can cause a reduction of gas exchange between plant and environment, with a consequently limitation of CO_2 assimilation and carbohydrate synthesis. In this research the influence of three irrigation levels (25, 50 and 100 % of maximum evapotranspiration restoration) on photosynthetic process in sorghum leaves was evaluate.

Two fiber sorghum genotypes (H_{133} and H_{952}) were sown in a split plot design with three replicates in which three irrigation levels treatments were applied: 25% (I_{25}), 50% (I_{50}) and 100% (I_{100}) of maximum evapotranspiration (ETm) restoration.

With regards to morphobiometric characters, plants reached the highest values of height in correspondence of maximum levels of irrigation and nitrogen fertilization (I_{100} N_{120}): observed value ranged between 451 and 484 cm respectively for H_{192} and H_{133}.

Dry biomass also showed significant variations in relation to the studied factors, with the highest value in thesis I_{100} N_{120} (3512 g m^{-2}). The level of irrigation I_{50} showed biomass production as much high, ranged between 2508 and 3120 g m-² respectively in I_{50} N_{60} e I_{50} N_{120}, against a production reduced by low irrigation in thesis without nitrogen treatments (986.7 g m^{-2}).

Both LAI and photosynthesis were higher in I_{100}, with significant differences among the three level of irrigation. The highest CO_2 assimilation activity was found in thesis with Etm restoration of 50 and 100%. In the mean of genotypes, the Leaf Area Index (LAI) ranged from 5.3 to 8.0 and the photosynthesis from 13.3 to 30.0 µmol m^{-2} s^{-1}. Significant differences were detected among genotypes in LAI: H_{952} showed higher values than H_{133}.

The higher photosynthetic activity in I_{100} can be attributable to the greater stomata number as consequence of the higher leaf area index (LAI).

Impiego della fluorimetria nel controllo della conservazione delle patate

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La patata (*Solanum tuberosum* L.) è una coltura alimentare di grande diffusione ordinariamente soggetta a conservazione dopo la raccolta. I produttori hanno l'esigenza di valutare i cambiamenti nei parametri qualitativi delle patate nell'arco del periodo di conservazione, dal momento che le transazioni commerciali correnti richiedono che i requisiti minimi di qualità vengano mantenuti costanti nel tempo. E' molto sentita l'esigenza di strumenti che permettano di monitorare le derrate in maniera non distruttiva.

In collaborazione con l'ERSA del Friuli-Venezia Giulia sono condotte da due anni prove di conservazione delle patate presso il CRA-IAA di Milano, per verificare l'applicabilità delle metodiche non distruttive disponibili alla valutazione dell'andamento del post raccolta.

I tuberi di patata qualitativamente idonei al consumo non contengono clorofilla (l'inverdimento comporta lo sviluppo di glicoalcaloidi tossici ed è pertanto una delle principali cause di scadimento qualitativo) ma possiedono un apparato fotosintetico potenzialmente attivo, il cui stato è legato alle condizioni fisiologiche. Si è quindi ritenuto che durante la conservazione i relativi valori potessero subire delle modificazioni. In effetti le misure hanno mostrato un andamento che, in diversi casi, riflette le condizioni di stress. Sono illustrati alcuni esempi di variazioni nei parametri fluorimetrici prossime al momento del germogliamento.

Intercalibration of fluorimeters and comparability of results

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This presentation reports the results of an intercalibration exercise carried out at Passo Pura (Udine) in June 2007. Five Handypea and 1 Mini PAM, provided from different working groups, participated in order to verify the comparability of the results. Before starting the exercise, each Handypea was fitted with the same measurement conditions (intensity of illumination: 3000 µmol m^{-2} s^{-1}; duration of illumination: 1 s; gain = 1). Each participant took the measurements on the same leaf clip, after 0 min of dark adaptation. Ten plants, with 4 leaf clips each, were taken into consideration. The results evidenced a quite high different sensitivity in recording the values of F_0 and F_M also among the Handypea fluorimeters (the coefficients of variation – CV - were 23% for F_0 and 20% for F_M), but the ratio between these values (F_0/F_M) was very less variable (CV = 8%). The quantum yield for primary photochemistry (F_V/F_M) was quite stable also considering Handypea and Minipam together (CV = 4.6%), as well as the parameters calculated on the normalized transient curves (only Handypea: V_J = 5.9%; V_I = 3.9%). The highest CV was detected for the Perfomance Index - PI_{ABS} – (30%), but only 12% if considering the Driving Forces (Log PI_{ABS}). Finally, an Handypea instrument gave regularly very different results respect to the others.

From an operational point of view, if different instruments are used in a same campaign of measurement, one should take in account that:

- The different instruments should be checked and compared before starting the campaign;
- F_V/F_M allows the better comparison even with different measurement conditions (different kind of instruments; different setting and so on);
- Normalized transients are less variable (and comparable) than the raw ones;
- Driving Forces allow a better comparison than the Performance Index.

Ozone stress on woody plants, detected by the chlorophyll a fluorescence transient

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This paper re-analyzes fluorescence data from open-top chamber (OTC) experiments, already published by different research groups, in order to individuate some general features of ozone stress on woody plants.

The experiments were carried out by Swiss and Italian research groups at the experimental research facilities of the Lattecaldo cantonal forest nursery (Switzerland, managed by The Swiss Federal Research Institute WSL, Birmensdorf) and the Curno regional forest nursery (Italy, managed by FLA, Milan and Catholic University at Brescia). The experimental settings were similar and consisted of four charcoal filtered air (CF) and four non-filtered air chambers (NF, where O_3 concentrations are about 92% in respect to ambient air). The woody species assessed were: *Fraxinus excelsior*, *Prunus avium* and *Viburnum lantana, Fagus sylvatica, Populus nigra* and *Quercus robur*. Ozone levels (AOT40 April-September) were close to 25 ppm.h at both sites, for all the considered years.

Chl *a* fluorescence transients of intact leaves were measured by means of direct fluorescence at different times during the seasons. On a logarithmic time scale, the rising transient from F_0 (when all the reaction centres of the PSII are open, i.e. when the primary acceptor quinone Q_A is fully oxidised) to F_P (where $F_P = F_M$ under saturating excitation light) had a polyphasic behavior. The analysis of the transient is called the JIP-test.

Ambient ozone concentrations lead to the closure of reaction centres (RC), which function as dissipater centers. All the parameters connected to dissipation were also increased. The quantum yield efficiency (F_V/F_M) demonstrated only little sensitivity. The response was not proportional to ozone exposition and/or fluxes. During the first part of the season, leaves were very resilient and photosynthesis could be transiently stimulated by ozone. Only towards the end of the growing season, efficiency and performance parameters showed a sudden drop. For species with terminated growth during the first part of the season, ozone has little effect on carbon fixation and storage. Growth was significantly reduced in species with a continuous growth pattern. The comparison of the shape of FT normalized per F_0 and F_M and per F_0 and F_J shows evident peaks at the steps K, J and I. Each peak corresponds to specific biochemical events. K indicates the reduced efficiency in the water splitting system. J indicates a Q_A accumulation in the single turnover region. I-peak seems to be more specifically connected to ozone stress due to the inactivation of Rubisco.

Use of fluorimetric and photosynthetic parameters to evaluate the effect of water stress conditions on *Photinia x fraseri* "Red Robin" and *Viburnum lucidum* development

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Fluorimetric and photosynthetic parameters are very useful to understand plant responses to drought stress. In ornamental plants, especially those utilized in Mediterranean dry urban landscape design, these parameters are very important because may allow to assess plant adaptability to drought conditions and thus to optimize water use. The aim of this research was to study the response of *Photinia* x *fraseri* "Red Robin" and *Viburnum lucidum*, two species used as broadleaf hedge shrubs, to water stress, by means of fluorimetric and photosynthetic parameters. Plants were grown into a greenhouse (placed in Pescia, PT, from June to October 2008), in pots filled with peat and pumice (1:1 v/v). Three levels of irrigation treatments were applied: 66%, 73% and 80% of container capacity (CC), with irrigations activated whenever the moisture content fell down to those values until restoration of the CC. In a fourth treatment, the moisture content was maintained constantly at 73%, by means of frequent irrigations of few seconds. Irrigation was regulated by an automated system interfaced with soil moisture FDR sensors.The following parameters were analyzed: transpiration, stomatal conductance, internal CO_2 concentration, net photosynthesis, water use efficiency (the photosynthesis/transpiration ratio), *chl a* fluorescence parameters (F_0, Fm, Fv/Fm, photochemical and non-photochemical quenching). Moreover, biometric parameters (leaf area, root growth, biomass accumulation, etc.) were also considered. Both physiological and growth data indicated that *Viburnum lucidum* was more sensitive than *Photinia* to water stress conditions. In fact, in *Viburnum*, significant differences in both fluorimetric and photosynthetic parameters were observed among treatments; the thesis with the higher moisture content showing the best results (irrigations at 80% of CC). Biometric data confirmed these observations. Otherwise, *Photinia* x *fraseri* "Red Robin" showed a better response to water stress treatments. In fact, the best results for all considered parameters were observed in the thesis where irrigation was activated when the soil moisture content was 73% of CC.

Effects of ozone on medicinal plants: the case of *Melissa officinalis*

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Ozone (O_3) is worldwide considered the major phytotoxic air contaminant. Sensitive plants exposed to ambient O_3 levels suffer significant growth reductions, premature senescence, as well as relevant alterations in secondary metabolism. Medicinal plants represent a field of interest poorly studied as far as phytotoxicity of air pollutants is regarded. So, we investigated the response of Melissa officinalis L. to O_3 pollution, under controlled conditions (i.e. fumigations in the form of a single square wave of 200 ppb for 5 h). Treated plants showed a wide range of physiological responses, like energy dissipation (photochemical quenching +40% and non-photochemical quenching -10%) and modifications in the pigment complex (increase of the ratio chlorophyll (*a*/*b*) of about 80%). After 48 hours it is possible to observe a macroscopical response, in the form of severe minute roundish dark-blackish necrosis, localized in the interveinal area of the adaxial surface of leaves.

Danni fogliari, efficienza fotosintetica e produttività in *Phaseolus vulgaris* esposto a ozono: uno studio basato sui flussi

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Gli elevati livelli di ozono a cui si trovano esposte le colture estive in Italia lasciano supporre l'instaurarsi di risposte di danno ad ogni livello di organizzazione biologica negli organismi vegetali coinvolti. A livello cellulare e subcellulare i danni si manifestano dapprima con alterazioni funzionali a carico dell'apparato fotosintetico e poi con lesioni strutturali delle membrane fino ad arrivare ad una vera e propria morte cellulare. Quando le lesioni interessano gruppi numerosi di cellule caratteristici sintomi necrotici diventano visibili sulla lamina fogliare. Più spesso le risposte rimangono sottotraccia dal momento che i sistemi antiossidanti e i meccanismi di riparazione contribuiscono a difendere l'integrità cellulare. Il costo energetico di tali processi si traduce però in un calo netto di produttività degli individui vegetali e di conseguenza in un minor raccolto.

Con l'obiettivo di quantificare gli impatti dell'ozono su una coltura orticola, piante di fagiolo cv. Borlotto Nano Lingua di Fuoco sono state allevate in 4 *Open Top Chambers*, due quali delle funzionanti con aria filtrata (dalle quali è stato rimosso O_3) e due con aria ambientale (con O_3).

Dopo l'emersione delle foglie primarie campioni fogliari sono stati prelevati regolarmente ogni tre giorni e sottoposti ad analisi microscopica istocitochimica al fine di individuare l'insorgenza di eventuali lesioni prima della comparsa di lesioni fogliari visibili. Contestualmente sono state effettuate misure di conduttanza stomatica, fluorescenza della clorofilla-a e riflettanza fogliare con l'obiettivo di: *i*) costruire un modello di conduttanza stomatica sulla base del quale calcolare la dose di ozono ricevuta dalle piante, e *ii*) rilevare alterazioni funzionali a carico dell'apparato fotosintetico e del ciclo delle xantofille. Al momento del raccolto gli effetti sulla produttività sono stati valutati attraverso l'analisi dei numeri e delle masse di baccelli e semi di ciascuna pianta.

Durante il periodo vegetativo le piante allevate in aria non filtrata hanno ricevuto una dose di ozono pari a 20.500 mmol O_3 m^{-3}, più del doppio di quelle allevate in aria filtrata. I sintomi fogliari visibili sono comparsi a partire da una dose critica di 1,33 mmol m^{-3}, preceduti 3-4 gg. prima da lesioni cellulari rilevabili microscopicamente. L'azione dell'ozono a livello subcellulare si è tradotta in una riduzione del contenuto di clorofille e dell'efficienza fotosintetica nonché nell'attivazione di meccanismi fotoprotettivi, tutti fortemente correlati alla dose ricevuta. Le ripercussioni sulla produttività si sono manifestate con una riduzione netta del numero di baccelli (-28%), che rivela anche un effetto inibente sulla fioritura, e di semi prodotti da ciascuna pianta (-33% in numero e -41% in massa).

Imaging PAM Fluorescence to evaluate virus attack in *Datura stramonium* L.

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Plant virus infections induce several physiological changes in the host that may lead to metabolic damages to cells, tissues and organs and to the expression of visible disease symptoms. Among the physiological processes, photosynthesis is heavily influenced by virus infections and the effects on the photosynthetic apparatus are mainly evident in the diseases that evolve through necrotic and chlorotic symptoms on aerial part of the plant host. Chlorophyll fluorescence imaging of virus-induced symptoms in plants has been previously reported for different plant-virus combinations and several studies indicated that viral infection alter chlorophyll fluorescence parameters.

In this work we used the chlorophyll fluorescence imaging to investigate the effects induced by *Tobacco mosaic virus* (TMV), *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) on the photosynthetic activity of *Datura stramonium* L., a widespread weed commonly used as test plant for virus biological indexing. The results indicated that the different plant-virus combinations induce different effects on electron transport process and on the plant photosynthetic activity, with unexpected positive effects induced by CMV. As usual, xanthophyll cycle was activated by the virus attack, again with different effects in CMV-infected plants. Chlorophyll fluorescence imaging seems to be able to provide an interesting tool for an early detection of photosynthetic stress in virus-inoculated plants. The study of the early stage of plant-virus interaction physiology is also feasible.

Photosynthetic responses of *Triticum durum* varieties to long-term ozone fumigation

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Two Italian wheat (*Triticum durum*) varieties, Mongibello and Claudio, were grown in fumigation chambers from emergence to maturity, where they were exposed to 80 ppb O_3 (7 days per week, h 10.00-15.00). From the tillering stage, "Mongibello" displayed a stronger reduction in photosynthetic activity in comparison to "Claudio" (-44.7 and -12.7%, respectively); in particular in "Mongibello" O_3 induced a significant increase in non photochemical quenching values (+80.0% in comparison to control): a higher portion of absorbed photons was lost as heat instead of being used to drive photosynthesis. During emergence of the inflorescence, only "Mongibello" showed a significant decrease in photosynthetic activity (-15.6%) and stomatal conductance (-33.7%), while "Claudio" showed a consistent decrease in chlorophyll (a and b) and β-carotene content. O_3 treatment induced a small reduction in F_v/F_m ratio (-2.8% in comparison to control). In "Mongibello", O_3 decreased the relative growth rate between inflorescence emission and maturity of caryopsis. As a result, the overall growth was clearly reduced with respect to controls.

Assessment of yield at harvest revealed a stronger O_3-induced decrease in the number of grains per ear in "Mongibello" with respect to "Claudio" (-40.0 and -28.6%, respectively). Only in "Mongibello" there was a decrease of the dry weight of grains per plant and of spikelets per ear. No negative effects on the dry weight of leaves and stems or in the number of ears per plant were found.

Our findings highlight the different response of the wheat varieties under study. The higher ozone sensitivity of "Mongibello" may be due to: (*i*) greater sensitivity in terms of growth during the critical period between inflorescence emission and maturity; (*ii*) decrease in photosynthetic activity from tillering to inflorescence emission; (*iii*) no exploitation as antioxidants of photosynthetic pigments.

Quenching Partitioning through light-modulated chlorophyll fluorescence: a quantitative analysis to assess the fate of the absorbed light in the field

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Plants use only 5-10% of the total absorbed energy for net carboxylation, while the remaining amount is dissipated via alternative strategies. involving thermal processes, fluorescence and photochemistry, all of which are competitive mechanisms. The main purposes of these pathways are the photosynthetic carboxylation and the dissipation of excess energy to limit the formation of reactive oxygen species (ROS) and photooxidative risks. Light-dependent thermal dissipation can be divided into photo protective quenching (accomplished by the xanthophyll cycle) and Photosystem II (PSII) photoinactivated re-emission; the latter's contribution is small because the complexes are quickly repaired and the detection of the real amount of the damaged PSII is made difficult by this effective and efficient recovery system. The remaining absorbed energy is engaged to mobilize the electrons through several photochemistry processes, including photosynthesis, photorespiration and the alternative electron transports.
The analysis of the commonly used fluorescence parameters gives a qualitative information about plant energy management, although it is difficult to appreciate the relative weight in energy partitioning of each pathway.

This study reports the application of quenching partitioning through a chlorophyll fluorescence approach performed on peach leaves subjected at three different light intensities (500, 1000 and 2100 μmol $m^{-2}s^{-1}$) for four times of exposure (30, 60, 90 and 120 minutes) in absence of photo-damage recovery. Furthermore, this methodology was compared with the $P700^{+}$ redox kinetic method for determining the functional PSII fraction in leaves.

In absence of recovery the active PSII concentration decayed with the photon exposure (the product between irradiance and the time of exposure) increase, following an exponential pattern according to the reciprocity law. The photoprotective thermal dissipation, ΦNPQ was proportional to irradiance up to 30 minutes of photoinhibitory treatment. Afterwards ΦNPQ was limited by the increasing competition for the absorbed energy re-emitted by the inactive PSII (ΦNF). ΦNF increased with the photon exposure dissipating up to 70% of the total incoming energy. The energy amount funneled to the photochemistry (ΦPSII) decreased with time of exposure and light intensity increase, becoming 0 after 120 minutes of photoinhibitory treatment at the maximal irradiance. The relation between the active PSII fraction, measured with the $P700^{+}$ redox kinetic method, and the energy amount used by the active PSII (1-ΦNF), deriving form the quenching partitioning, was linear.

The quenching partitioning trough light modulated chlorophyll fluorescence is a useful tool to analyse plant energy management and gives also a good estimation of the active PSII fraction. This methodology can be easily used in the field as measurements are rapid and not destructive and the detection devices are becoming more and more portable.

Valutazione dell'efficienza fotosintetica in risposta a stress da zinco nelle Pteridofite

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Il presente studio è stato condotto in sistema idroponico, utilizzando come medium la soluzione Hoagland's ½ strength, pH 5.9, addizionata di Zn 0, 50, 125, 250 e 500 mg L^{-1} ($ZnSO_4$). La risposta fisiologica allo stress indotto dallo Zn nelle felci *Polypodium cambricum* e *Pteris vittata* è stata valutata come variazione temporale dell'efficienza fotosintetica. *P. vittata* è una nota iperaccumulatrice di As, che tollera e accumula nelle parti aeree anche discrete quantità di Zn crescendo in siti co-contaminati (1, 2), mentre *P. cambricum* accumula Zn prevalentemente a livello ipogeo (3).

Le variazioni dell'efficienza fotosintetica sono state analizzate attraverso misure di fluorescenza del fotosistema II, realizzate con il fluorimetro diretto Handy-PEA. Durante il periodo di esposizione (3 settimane), la fluorescenza della clorofilla *a* è stata misurata su 3 fronde (pinnula basale, apicale e mediana) per trattamento, con cadenza temporale di due volte a settimana.

L'analisi della curva di induzione della fluorescenza (JIP test) e dei relativi parametri della fisiologia fogliare, secondo Strasser *et al*. (4), è stata integrata con il monitoraggio sia dei danni macroscopici che anatomici a livello dei diversi tessuti e organi esaminati.

L'efficienza fotosintetica delle piante sottoposte a trattamento con Zn 50 e 125 mg L^{-1} non ha evidenziato decrementi significativi rispetto alle piante controllo (Zn 0). Viceversa, i trattamenti Zn 250 e 500 causano una riduzione della capacità fotosintetica. Il decremento della massima emissione di fluorescenza (Fm) si riscontra alla concentrazione Zn 250, in quanto a Zn 500 si manifestano danni più estesi e generalizzati, che causano in breve tempo la morte delle piante.

L'analisi della fluorescenza fogliare in piante esposte a xenobionti può rappresentare un valido strumento per determinare e quantificare il loro stato di stress, anche prima del manifestarsi di sintomi visibili.

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Productivity & Photochemical Performance of Mass Microalgae Cultures in Outdoor Units

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In outdoor inclined-surface cultivation systems for microalgae, the suspension flows over a cascade of sloping planes in a thin layer (~ 6 mm) exposed to solar irradiance making it possible to reach high biomass density – up to 45 g dry weight L^{-1}. These units are characterised by the high ratio of exposed surface-to-total volume of >100 m^{-1} (as compared to open ponds ~10 m^{-1}) and highly turbulent flow which enables high volumetric and areal productivity. The aim of this work was (*i*) to correlate photochemical activity and productivity, and (*ii*) to determine biomass density range for high-productivity photo-optimised cultures. Dissolved oxygen concentration, pH, biomass density, pigment content, and productivity were measured in the cultures. Chlorophyll fluorescence was monitored *in-situ* (using saturation-pulse method) to calculate F_v/F_m, and $\Delta F/F_m$' (fluorometers PAM 101-103 and PAM-2000, H.Walz). Fast fluorescence induction curves were measured in microalgae cultures to record the transient phases denoted as O, J, I and P (Kautsky curves) which reflect electron transport processes in the photosynthetic membrane at molecular level (fluorometer Fl-2000 and AP100, P.S.I. Brno). Photochemical activities and growth parameters were studied in *Chlorella sp.* cultures of biomass density between 1 and 47 g L^{-1}. The Chlorella biomass grown in the thin-layer cultures contained relatively high content of carotenoids up to 0.5% of total biomass; the major compounds being xanthophylls – lutein (about 75%), violaxanthin (about 7%) and neoxanthin (about 4%). Fluorescence measurements showed that diluted cultures (1-2 g DW L^{-1}) experienced significant photo-stress due to inhibition of electron transport in the PSII complex. The presence of zeaxanthin (a mean of energy dissipation in the so-called xanthophyll cycle) and high Car/Chl ratio indicated excess irradiance in some cultures. The highest photochemical activities were achieved in the cultures of 5-15 g dry weight L^{-1}, which performed the maximum daylight productivity up to 7 g biomass m^{-2} h^{-1}. In these cultures, a midday-depression of F_v/F_m between 20-30% as compared with morning values, proved to be compatible with well-performing cultures. The increased J peak of the fluorescence induction curves in *Chlorella* cultures indicated that electron transport is slowed-down at the acceptor side of the PSII complex ($Q_A \rightarrow$ PQ pool). We concluded that:

- Chlorophyll fluorescence monitoring is a suitable technique to monitor photochemical performance of mass microalgae cultures – fast, non-invasive and easy to measure.
- In outdoor microalgae cultures, the cells are photo-acclimated to an averaged light intensity which is related to ambient irradiance, cell density and culture layer thickness. The turbulent flow is necessary to allow rapid light/dark cycles in hundreds of microsecond to milliseconds to match the turnover of the photosynthetic apparatus. The optimal combination of these factors is crucial for high productivity.
- A midday depression of the F_v/F_m ratio of about 20-30 % does not cause a reduction in the productivity in well mixed microalgal cultures. The lower or higher depression of F_v/F_m indicated shade-adapted or photoinhibited cultures, respectively.

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Are ecophysiological and histochemical responses to ozone in *Fraxinus* species influenced by the provenance?

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It is well known that the responses of trees to air pollutants vary between and within species under strong genetic control, but this phenomenon can be also regulated by plant provenance. Leaf symptoms attributed to ozone (O_3) have been detected in a growing list of tree species such as ash, commonly planted in urban area and also important forest trees in Italy. Two-years old seedlings of *Fraxinus ornus* and *F. excelsior*, represented both by two provenances (Piedmont e Tuscany) were exposed to O_3 fumigation (150 ppb for 8 h d^{-1}, 35 d) and compared to controls maintained in filtered air. Treated *F. excelsior* developed chlorosis and adaxial necrosis on mature fully expanded leaves. This was true for both provenances even if different photosynthetic efficiency was observed. O_3 directly influenced in some cases stomatal aperture, because stomata are closed prematurely, inducing a slow CO_2 movement into the leaf; in other cases, stomatal closure resulted as disturbances in the photosynthetic apparatus. In Piedmont provenance, after only 7 days of treatment, O_3 induced a small decrease in F_v/F_m ratio (-5.2% in comparison to controls), as a consequence of a significantly increase in minimal fluorescence value (+15.3% in comparison to controls). In Tuscany provenance, after 14 days of treatment, it is observed a different physiological response of treated plants, like energy dissipation (photochemical quenching -10.3% and non-photochemical quenching -14.9%, in comparison to control). *F. ornus* showed variations between the provenances: trees from Tuscany were generally more tolerant to O_3 in terms of injury than those from North Italy. The F_v/F_m ratio did not significantly change during the experiment, but only after 14 days of treatment with a small reduction (-5.2 and -5.5% respectively in Piedmont and Tuscany provenance, in comparison to controls). Only after 21 days of treatment, O_3 induced an increase in non-photochemical quenching values in Tuscany provenance (+5.8% in comparison to control): a higher portion of absorbed photons was lost as heat instead of being used to drive photosynthesis. Histochemical markers such as autofluorescence, the presence of cell-wall thickenings, the enlarged cell vacuole and the typical necrotic lesions of palisade tissue were investigated in order to understand if symptoms induced by O_3 allow differential diagnosis of O_3 injury. Moreover the final purpose of this work was to analyze species-specific and provenance-specific antioxidative capacity and to compare the antioxidant capacity of symptomatic (O_3 visible-injury) and asymptomatic (O_3-symptom free) leaves.

Photosynthetic behaviour of *Liriodendron tulipifera* and *Tilia americana* exposed to chronic ozone fumigation

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The photosynthetic response to ozone (O_3) was studied in seedlings of two ornamental trees, *Liriodendron tulipifera* and *Tilia americana*, respectively considered O_3-sensitive and O_3-resistant, after exposure to the pollutant for 45 consecutive days (120 ppb, 5 h d^{-1}). The species showed a different phenomenological response: only *L. tulipifera* developed necrosis on fully expanded leaves, at the end of the treatment. O_3 decreased the CO_2 assimilation rate at light saturation level (-33%) and the stomatal conductance (-6%) from day 8 on. The reduction of photosynthetic rate was mainly due to a reduced mesophyllic activity, as evidenced by the increase in intercellular CO_2 concentration (+8%). Only after 30 days of exposure, *T. americana* showed a limitation of the photosynthetic processes, not only due to stomatal components. In *L. tulipifera* alterations of chlorophyll fluorescence parameters, in particular the minimal fluorescence level (F_0), the maximal fluorescence level (F_m) and the variable and maximal fluorescence ratio F_v/F_m were observed from day 8 until the end of exposure. In *T. americana*, instead, O_3 caused a decrease of F_v/F_m only at the end of the treatment. These results indicate the different response of these species and a higher O_3 sensitivity of *L. tulipifera*, as evaluated by visual symptoms and by a greater impairment of the photosynthetic processes.

Influenza dell'ammoniaca sull'emissione di fluorescenza clorofilliana nei licheni

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L'ammoniaca (NH_3) rappresenta la principale fonte di deposizioni azotate intorno agli allevamenti di bestiame e come gas può avere effetti sulla struttura e composizione delle comunità vegetali. Anche le comunità licheniche ne risultano influenzate e possono pertanto rappresentare dei buoni indicatori delle conseguenze dell'esposizione all'NH_3 in fase gassosa. Alcuni effetti sono individuabili anche precocemente a livello fisiologico. In tal senso, è stato realizzato in Grecia un esperimento di esposizione in campo della durata di un mese di talli delle specie *Evernia prunastri* e *Pseudevernia furfuracea* (due licheni frequentemente utilizzati in studi di biomonitoraggio) a distanza crescente da un allevamento di ovini selezionato allo scopo. La distanza dall'allevamento coincide con un decremento delle concentrazioni di NH_3 in atmosfera registrate con campionatori passivi. Infatti, presso la sorgente di emissione è stato misurato un livello di NH_3 in atmosfera pari a circa 60 $\mu g/m^3$, che scende a 15 $\mu g/m^3$ a 60-80 m dalla fonte. Le curve dell'emissione di fluorescenza clorofilliana nei campioni esposti sono state analizzate per valutare eventuali effetti dell'NH_3 sul processo fotosintetico confrontando i dati con quelli dei campioni prima del trapianto.

L'esposizione alla fonte di NH_3 ha avuto conseguenze negative rilevabili in entrambi i bioindicatori già dopo 15 giorni di esposizione in funzione della distanza e corrispondenti a un drastico peggioramento della performance fotosintetica.

I talli lichenici trapiantati in corrispondenza dell'allevamento hanno mostrato un notevole decremento dei valori di efficienza fotosintetica, così come indicata dal rapporto F_V/F_M, l'indicatore di risposta applicato più comunemente. L'analisi di alcuni parametri derivati dal JIP-test ha evidenziato l'alterazione di diversi target del processo fotosintetico per l'esposizione all'NH_3: nei campioni trapiantati alla distanza minore dall'allevamento risulta evidente una diminuzione dell'attività di trasporto degli elettroni e della sua efficienza, la riduzione della densità dei centri di reazione attivi e quindi un corrispondente aumento della frazione di energia che essi devono assorbire, nonché l'aumento della frazione di energia dissipata rispetto a quella assorbita. Al termine dell'esposizione, specialmente in *P. furfuracea*, è stata rilevata una diminuzione superiore al 75% dell'indice globale della performance fotosintetica rispetto ai valori misurati nei campioni prima del trapianto. Non bisogna tralasciare il fatto che in ambiente Mediterraneo, il clima ha un ruolo notevole nel regolare la fotosintesi: nell'interpretazione dei dati bisogna tener conto dell'influenza della disponibilità idrica sul metabolismo lichenico e pertanto del ruolo che l'aridità estiva può svolgere nel determinare gli adattamenti del processo fotosintetico. È importante in tal senso riuscire a delineare gli effetti dovuti a un inquinante rispetto a quelli determinati dalle condizioni climatiche o da altre caratteristiche intrinseche dell'area di studio.

Populus maximowiczii x *P.* x *berolinensis*: a model tree to study ozone stress

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Populus maximowiczii Henry x *P.* x *berolinensis* Dippel (short name: 'Oxford clone') has been widely used in open-top chamber experiments in Southern Switzerland (Lattecaldo) and Curno (Northern Italy). This clone is also used as a biomonitoring tree species within the UNECE/ICP-Forests monitoring network. Results from the OTC experiments have been published in several papers revealing the characteristics of a fast growing and highly ozone sensitive poplar clone. The performed studies aimed to determine (*i*) the levels and doses of tropospheric ozone that cause the onset of foliar symptoms, (*ii*) to gain a better understanding of dynamics of symptom development within the crown with emphasis on possible compensation mechanisms of photosynthesis across the different parts of the crown., (*iii*) to detect signals of pre-visual damage applying morphological and physiological methods, (*iv*) to detect possible changes in the carbon isotopic composition of the wood biomass, and (*v*) to assess the reduction of growth in relation to the above described physiological and morphological effects occurring under natural field conditions and ambient ozone concentrations.

The present paper compares the results of the different studies in order to gain a comprehensive understanding of the behaviour of the respective poplar clone. Most recent findings suggest that this poplar clone may have very suitable characteristics for a broad leaf model tree for ozone risk assessment.

Economic or environmental aspects should also be considered, in particular in respect to the extended poplar plantations managed in the Po Valley (Northern Italy) as well as in respect to the important role poplar may play in the production of fuel biomass. Unlike the findings from natural adult woodland studies (where the ozone effect on growth has been found to be minor or absent), poplar cultivations seem to be at high ozone risk.

AirFLEX airborne sensor: remote sensing to quantify photosynthetic efficiency by measuring sun-induced fluorescence in the oxygen absorption bands

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Questo lavoro si inserisce nel contesto della ricerca effettuata durante le campagne di misura CEFLES2 (CarboEurope, FLEx and Sentinel-2) nell'ambito del progetto Carbo Europe Regional Experiment Strategy e a supporto del progetto FLEX, candidato al Core Earth Explorer Mission Ideas (ESA).

L'oggetto della ricerca riguarda l'impiego di tecniche innovative di telerilevamento da piattaforma aerea volte all'osservazione del processo fotosintetico attraverso la stima della fluorescenza della clorofilla indotta dal sole (SIF) utilizzando un sensore sperimentale AirFLEX installato su di un velivolo Piper Seneca III. La campagna di acquisizione è stata organizzata in modo tale da eseguire 14 voli nella zona delle Lande (Francia) in tre periodi diversi (Aprile, Giugno, Settembre 2007) e in finestre temporali tali da coprire l'intera fase diurna. AirFLEX ha permesso la restituzione di un elevato numero di prodotti includendo le radianze a 687 e 760 nm, la fluorescence fraction, il PRI (Photochemical Reflectance Index) e l'indice NDVI (Normalized Differential Reflectance Index). Una camera nell'infrarosso termico (Flir) ha inoltre acquisito informazioni relative alla temperatura superficiale sincronizzate alla fluorescenza telerilevata. Un secondo velivolo SkyArrow ERA è stato impiegato per la misura del vento e della turbolenza, flussi di CO_2, radiazione netta, incidente e riflessa.

Le misure radiometriche e i flussi di CO_2 acquisiti hanno permesso di approcciare un'analisi al fine di valutare le potenzialità di quantificare l'efficienza fotosintetica e il GPP (Gross Primary Production) da misure passive di fluorescenza.

Valutazione dello stress post-produzione delle piante fiorite in vaso attraverso la misura della fluorescenza della clorofilla a

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Le piante fiorite in vaso durante la fase di post-produzione trascorrono spesso diversi giorni in ambienti con scarsa illuminazione e temperatura spesso non ottimale. La mancanza di un periodo adeguato di acclimatazione comporta una rapida perdita delle caratteristiche ornamentali e commerciali. Questo lavoro si propone di individuare un sistema per il monitoraggio della qualità di alcune specie ornamentali da vaso fiorito spesso destinate all'esportazione durante il periodo di post-produzione come buganvilla, lantana e ibisco. Le piante sono state conservate al buio a 14 °C, per simulare il trasporto e successivamente sono state trasferite per tre giorni in camera di crescita a 20 °C per valutare il recupero dallo stress prima della vendita.

La fluorescenza della clorofilla *a* è stata misurata all'inizio, al termine della conservazione e durante il periodo di recupero. Contemporaneamente sono stati misurati i livelli endogeni di acido abscissico e la produzione di etilene dai fiori e delle foglie. Inoltre, è stata valutata la correlazione tra i livelli dei due ormoni e i valori di fluorescenza.

La fluorescenza della clorofilla a si è rilevata un buon marcatore dello stato di stress e della qualità postproduzione delle piante ornamentali fiorite in vaso.

* * *

Evaluation of post-production stress in flowering potted plants by chlorophyll a fluorescence

Flowering potted plants during the post-production stage are usually stored in environments with low light intensity and temperature often inadequate. The lack of an acclimatization period may affect the ornamental and commercial quality.

The aim of this work was the identification of a system for monitoring the quality of several ornamental species destined to export such as Bougainvillea, Lantana and Hibiscus. Plants were stored in dark at 14 °C for simulating the transport conditions and subsequently were transferred for three days in growth chambers at 20 °C for evaluating the recovery before selling.

Chlorophyll a fluorescence was measured in all plants at beginning, at the end of storage and during the recovery period. Contemporary ethylene production and abscisic acid were determined in leaves and flowers. Correlation analysis between plant hormone levels and fluorescence values were calculated. The chlorophyll a fluorescence resulted a good stress marker and a sound tool for evaluating commercial quality of lowering ornamental potted plants.

Annual course of the maximum PSII efficiency Fv/Fm of Mediterranean *Quercus* species of a model plantation in central Europe

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According to climate change scenarios the central European climate will change to dryer and hotter conditions within the next 100 years. On today already dry stands, the predominant beech (*Fagus sylvatica*) will be replaced in the long run by more drought tolerant tree species. *Quercus* species will probably be more abundant on these arid sites. Even the Central European oak species like *Quercus robur* could have problems to rise under such severe climate conditions.

Therefore different oak species from the Mediterranean as well as the central European *Quercus robur* are planted already today in a model plantation under central European climate conditions in Frankfurt am Main, Germany. Two different water regimes are tested, one control with the normal precipitation and one simulating the severe water conditions in central Europe of the year 2003. Both subplots are exposed to the temperatures (also hard winter conditions) of the stand in Frankfurt am Main.

Chlorophyll fluorescence parameters were used to test the ability of different *Quercus* species to grow under central European climate conditions of today as well as with different water regimes.

Our measurements showed that Mediterranean oak species like *Quercus ilex* can grow in Central Europe, even with the still cold a and freezing winter conditions in Germany of today.